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THE CATALYTIC DECOMPOSITION OF GLUCOSE IN AQUEOUS SOLUTION BY THERMAL PROTEINOIDS

UNPUBLISHED PRELIMINARY DATA

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S. W. Fox and G. Krampitz, Nature 203, 1362 - 1364 (1964).

The thermal condensation in an initially dry state, of the eighteen amino acids common to protein [1,2,3], represents a synthesis by which protein molecules can be visualized as having come into existence simply in a geological matrix [4]. These thermal polymers, proteinoids, readily form membranous microparticles [5,6], have nutritive quality for bacteria [3] and rats [7,8], are split by proteolytic enzymes [2,3], and have their own catalytic activity for the hydrolysis of p-nitrophenyl acetate [9], in some cases at a rate many times that observed with the amount of histidine contained in the polymer [9,10].

Preliminary reports of activity for the hydrolysis of ATP at 40° by zinc-containing proteinoids are appearing [11,12]. In the present communication is reported the catalytic activity in aqueous solution of thermal proteinoids for the decomposition of the natural substrate, D-glucose.

The activity observed concerns the decomposition of uniformly labelled C^{14}-glucose to $C^{14}O_2$ at a low rate of conversion; some $C^{14}O_2$ production has however been measured in every one of more than sixty experiments. Glucose labelled only in the 6-position showed a comparable rate of production of $C^{14}O_2$, whereas glucose labelled in the 1- or 2-position yielded virtually no $C^{14}O_2$. Evidence has been obtained that one of the intermediates is glucuronic acid, and radio-glucuronic acid itself has been found to yield $C^{14}O_2$ in the presence of thermal proteinoid, at a rate many times that of the production from glucose.

In a typical experiment, 100 mg of a 2:2:1-proteinoid [3/] dissolved in 50 ml of redistilled water was incubated at 37.5° for 72 hrs. with 10 μC of uniformly labelled C^{14}-glucose (specific activity of 28.5 mC/mM; Calbiochem) freshly purified by chromatography. The reaction mixture was maintained under a slow stream of prepurified nitrogen and the gas was passed through a flask containing saturated barium hydroxide. The CO_2 traps were replaced after 1, 2, 3, 6, 12, 24, 48, and 72 hrs. To each trap was added 50 mg of carrier $BaCO_3$; the total carbonate was collected on Whatman no. 50 filter paper after complete precipitation. The precipitate was washed with 50 ml of water and then 20 ml of ethanol. The radioactivity was estimated on a windowless, gas flow beta-detector operated in the Geiger range with helium-isobutane. The counts were determined over 10 min. Controls without proteinoid and others without glucose were run. The results of studies of evolution of carbon dioxide are represented in Table I.

TABLE I

Evolution of $C^{14}O_2$ from Uniformly Labelled Glucose (10 μC) on Incubation with Thermal Proteinoid

Proteinoid or Control	$C^{14}O_2$ produced in c.p.m. [a]	Standard Deviation
2:2:1 (DJ IV 51)	117	5
2:2:1, microspheres	64	7
2:2:1 (DJ V 15)	70	9
2:2:1 (190° RM)	62	6
1:1:1-Zn (BW IV 79B)	79	5
1:1:1-Zn, purified from hot H_2O	68	5
1:1:1 (GK I 40)	83	5
Lysine proteinoid (KS IV 23 b, d)	1023, 1278	25, 36
2:2:1 (GK aseptic)	441	13
Amino acid mixture	0-5	0
Glucose in solution	0-5	0
Hydrolyzate of DJ IV 51	0-5	0

[a] Each value is the figure above background (42-55 c.p.m.).

Mixtures of amino acids in the proportions found in the analysis of the proteinoid were observed to be devoid of catalytic activity, as were direct hydrolyzates (Table I).

Some increase in rate of evolution of carbon dioxide was observed when ATP or Mg^{++}or both were added to the reaction mixture (Fig. 1). The shape of the curves constitutes part of the evidence ruling out microbial contamination as a source of enzymic activity. Most of the experiments were at pH 2.5, which of course inhibited most or all microbial growth. As a rigorous check on the possibility of contemination, syntheses were carried out aseptically under the supervision of Dr. and Mrs. Lutz Wiese.

The aseptic syntheses began with amino acid mixtures which had stood under diethyl ether for 48 hr and which had no catalytic activity per se. The acid proteinoids thus prepared were tested for sterility in tryptone glucose beef extract, a) broth and b) agar, in tryptone glucose yeast extract, c) broth and d) agar, each before dialysis of the proteinoid, after dialysis for 4 days, after re-solution of aseptically lyophilized polymer and at the end of the tests for catalysis. The polymers thus prepared showed at least the usual amount of activity after having given negative results for growth in the sterility tests. Lysine proteinoid was also synthesized under sterile conditions. Both the aseptically synthesized lysine proteinoid and lysine proteinoid prepared months earlier, and stored in the usual way in a bottle, were found to be bacteria-free;

this latter probably reflects the bacteriostatic activity of polylysine [13].

As Table I shows, thermal lysine proteinoid [9], as first prepared by Dr. Harada [9], was more active than thermal acid proteinoid.

Other sugars, radio (fructose) and radio (sucrose), also yielded $C^{14}O_2$ but in smaller proportions than did (radio) glucose. In three experiments, 10 μC of sucrose yielded 130, 212, and 316 c.p.m. Such results with sucrose help to rule out the possibility that decarboxylation is due to a Maillard reaction between proteinoid and the sugar [14]. Study of the pH-activity curve for lysine proteinoid also revealed pH optima at 2 and 5, whereas the Maillard reaction is favored in alkaline solution [14]. The proteinoids were active over the entire range of pH 1-9 tested. Activity was only slowly destroyed by heating in aqueous solution. Microspheres [5,6] formed in the steam autoclave from hot water also displayed activity for the evolution of carbon dioxide. An experiment in which a second charge of substrate, added after three days, was decomposed as rapidly as the first charge represents one experiment to test whether the activity is truly catalytic.

For analytical purposes, the reaction mixture was concentrated in a rotary evaporator and finally dried in a vacuum desiccator over sodium hydroxide pellets and over sulfuric acid. The dried residue was thrice extracted with dry pyridine in order to remove unreacted glucose; the pyridine extract was taken to dryness over sulfuric acid.

Aqueous solutions of the residue from pyridine extract and from the pyridine-insoluble residue were separately subjected to paper chromatography. The samples were applied to Whatman 3MM paper. The developing solvent usually was ethyl acetate: pyridine: water (12:5:4). Chromatography was carried out by ascension (Fig. 2). Fig. 2 presents an initial depiction of the results.

Following the suggestion of glucuronic acid as an intermediate from the experiment with 6-C^{14}-glucose, this acid was sought as follows. The reaction mixture was made alkaline to phenolphthalein with barium hydroxide. Carbon dioxide was bubbled through this mixture for a few minutes. The mixture was then heated to 100° for 10 min., filtered, and the ppt was washed with boiling water. The combined filtrates and washings was concentrated to 2 ml in vacuo and mixed with 100 ml of ethanol. The barium salts were collected by filtration and washed with ethanol. The radioactive ppt was acidified with acetic acid in aqueous suspension and the barium was removed with 1 N sulfuric acid, the liquid dried, and extracted with ethanol. The extract was subjected to two dimensional paper-chromatography [15] with phenol: H_2O (100 g:39 ml) in the first direction and butan-1-ol: propionic acid: water in the second direction. (For this chromatography, two solutions were prepared. Solution A contained 919 ml of butan-1-ol and 81 ml of distilled water. Equal volumes of solutions A and B were mixed just before use.) One of the spots had the R_f of glucuronic acid as determined with a

control. The material was eluted and rechromatographed in ethyl acetate: pyridine: water (120:50:40). The R_g (R_g = $\frac{\text{distance substance travels from origin}}{\text{distance glucose travels from origin}}$ X 100) of the sample corresponded with that of glucuronic acid, was 13-14. This eluted spot was rechromatographed in methanol: formic acid: water (30:15:5 v/v), again indicating glucuronic acid.

From an experiment on 3.0 g of glucose (100 mg of proteinoid), the isolated glucuronic acid fraction was subjected to reaction with carbazole [16] to give a positive color test. The maximum absorbance was at 530 mμ, the same maximum being found with the carbazole reaction product of authentic glucuronic acid.

In another reaction of 100 mg of 2:3:1-proteinoid, 10 g of D-glucose and 10 μC of U-C-14 glucose the extract, following barium sulfate formation and ethanol extraction, was dried in a desiccator. The residue was dissolved in 10 ml of 0.2 N sodium acetate and 100 mg of p-bromophenylhydrazine hydrochloride was added. The suspension was heated to boiling for 5 min. and 50 mg of authentic non-radioactive glucuronic acid p-bromophenylhydrazone was added. The suspension was chilled for 2 days. The solid was filtered, dissolved in hot 60% ethanol and left in the refrigerator for 48 hr. This recrystallization was repeated 4 times. The yellow needles m.236°C unc. [17], mixed m.p. 236°C unc. When examined in a planchet with a windowless counter, 3700 c.p.m. were recorded. Following a sixth recrystallization, 3700 c.p.m. were again recorded.

The radioactive p-bromophenylhydrazone was chromatographed in water saturated with butan-1-ol. The R_f for this material on a radioautogram and for cold material observed visually was in each case 0.89. A mixed chromatogram showed also $R_f = 0.89$.

The evidence thus indicates that at least a small fraction of the glucose is first converted to glucuronic acid, which is then decarboxylated, and that each of these two reactions is catalyzed in aqueous solution by proteinoid prepared by thermal condensation of dry amino acids mixed in appropriate proportions. Fig. 2 is a radioautogram which is part of the evidence that the glucose is over 90% converted to other materials.

These results may explain many earlier findings in this laboratory in which were observed activities for other natural substrates; the variability of such results since 1958 was such that no report has been submitted for publication. This variability now appears to be explainable in part as due to the presence of several weak activities. The evolutionary value of weak catalytic activities, such as compared to those of contemporary enzymes and as here demonstrated for production of polymers active in producing carbon dioxide and glucuronic acid, has been spelled out in theoretical treatments particularly by Calvin [18].

The finding of catalytic activities for natural substrates and of integrated nutritive qualities in thermal proteinoids entitles them to be considered both as synthetic

general protein and as a model of primitive abiotic protein. In the latter context a catalytic proteinoid which forms organized units [4-6] is conceptually consistent with an evolution which led to diversified, specialized, and quantitatively enriched catalysts.

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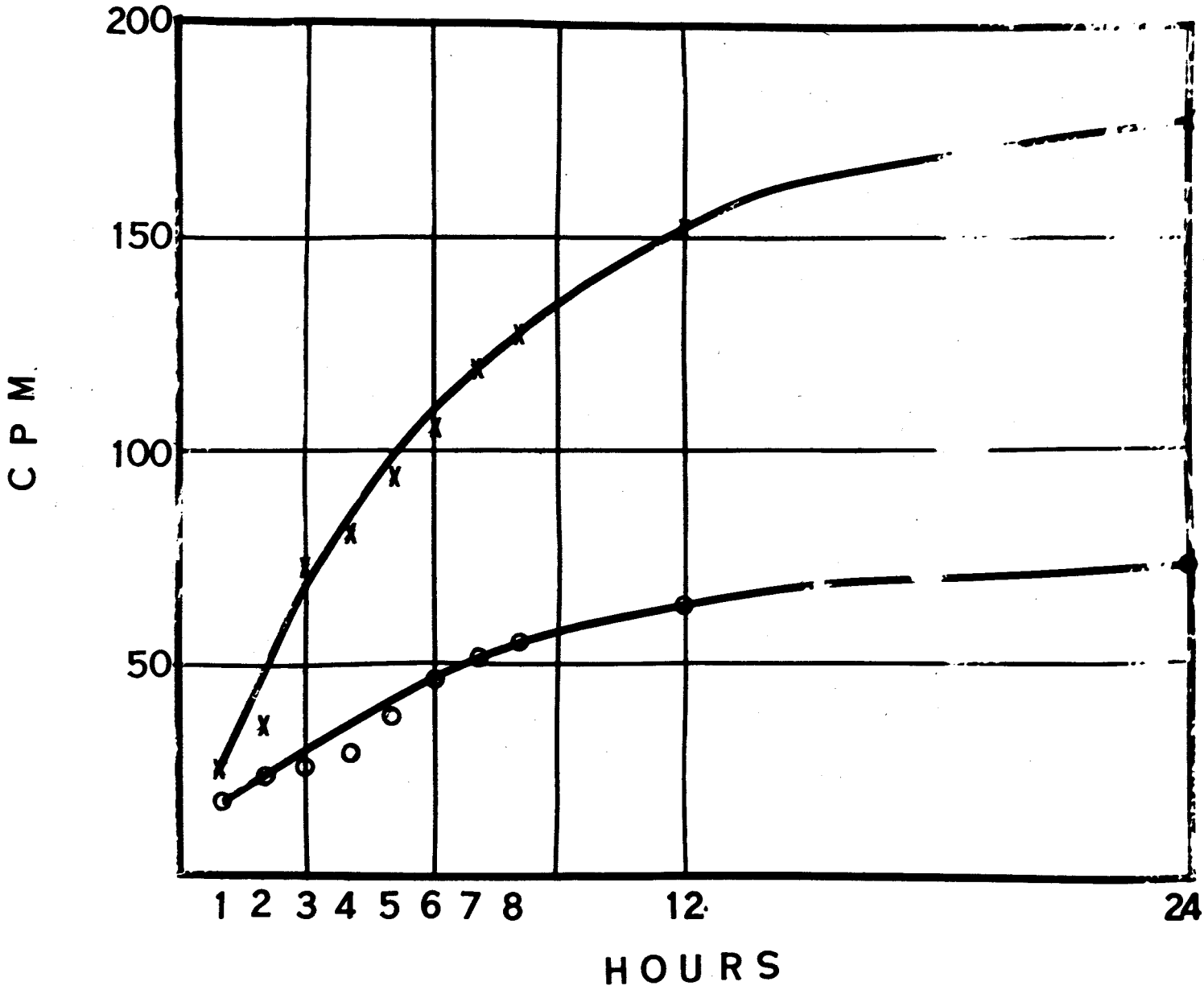

Fig. 1. Evolution of $C^{14}O_2$ from radioglucose in presence of thermal proteinoid. Fig 1 demonstrates the carbon dioxide formation from glucose in the presence of proteinoid. O————O shows carbon dioxide evolution for the reaction mixture without any additions. X————X indicates carbon dioxide production from the reaction mixture in the presence of 2.5 μM ATP.

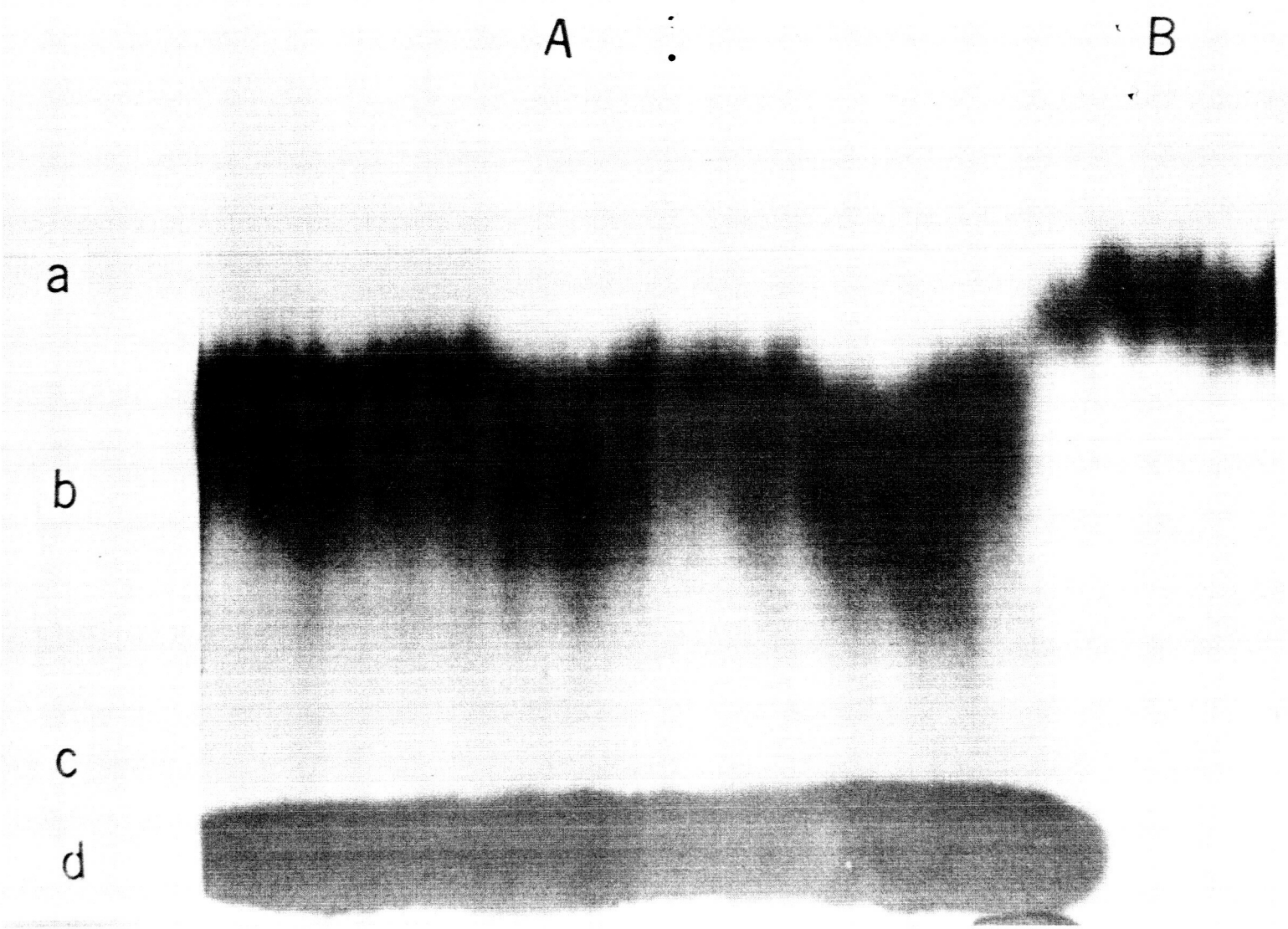

Fig. 2. Radioautogram of decomposition of radioglucose catalyzed by thermal proteinoid. Fig. 2 demonstrates the decomposition products of U-C-14 glucose, after incubation with a lysine proteinoid, in a solvent system of ethyl acetate: pyridine: water (12:5:4) ascending technique; Whatman no. 3 MM paper. A is the water soluble extract of the reaction mixture. B is the water soluble extract of the reaction mixture before incubation. Bar a corresponds to glucose, b is unidentified, but does not contain free glucose after rechromatography since it is insoluble in pyridine. On rechromatography, R_f of glucose = 0.27, R_f of major spot = 0.22. Bar c has R_f identical with that of glucuronic acid after rechromatography in other solvent systems. Bar d is unknown, contains no free glucose nor free glucuronic acid.